



Abboud, M. I., Kosmopoulou, M., Krismanich, A. P., Johnson, J. W., Hinchliffe, P., Brem, J., Claridge, T. D. W., Spencer, J., Schofield, C. J., & Dmitrienko, G. I. (2017). Cyclobutanone Mimics of Intermediates in Metallo- β -Lactamase Catalysis. *Chemistry - A European Journal*.
<https://doi.org/10.1002/chem.201705886>

Publisher's PDF, also known as Version of record

License (if available):
CC BY

Link to published version (if available):
[10.1002/chem.201705886](https://doi.org/10.1002/chem.201705886)

[Link to publication record in Explore Bristol Research](#)
PDF-document

This is the final published version of the article (version of record). It first appeared online via Wiley at <https://doi.org/10.1002/chem.201705886> . Please refer to any applicable terms of use of the publisher.

University of Bristol - Explore Bristol Research

General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available:
<http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

Enzyme Catalysis | Hot Paper |

Cyclobutanone Mimics of Intermediates in Metallo- β -Lactamase Catalysis

Martine I. Abboud,^[a] Magda Kosmopoulou,^[b] Anthony P. Krismanich,^[c] Jarrod W. Johnson,^[c] Philip Hinchliffe,^[b] Jürgen Brem,^[a] Timothy D. W. Claridge,^[a] James Spencer,^{*,[b]} Christopher J. Schofield,^{*,[a]} and Gary I. Dmitrienko^{*,[c]}

Abstract: The most important resistance mechanism to β -lactam antibiotics involves hydrolysis by two β -lactamase categories: the nucleophilic serine and the metallo- β -lactamases (SBLs and MBLs, respectively). Cyclobutanones are hydrolytically stable β -lactam analogues with potential to inhibit both SBLs and MBLs. We describe solution and crystallographic studies on the interaction of a cyclobutanone penem analogue with the clinically important MBL SPM-1. NMR experiments using ^{19}F -labeled SPM-1 imply the cyclobutanone binds to SPM-1 with micromolar affinity. A crystal structure of the SPM-1:cyclobutanone complex reveals binding of the hydrated cyclobutanone through interactions with one of the zinc ions, stabilisation of the hydrate by hydrogen bonding to zinc-bound water, and hydrophobic contacts with aromatic residues. NMR analyses using a ^{13}C -labeled cyclobutanone support assignment of the bound species as the hydrated ketone. The results inform on how MBLs bind substrates and stabilize tetrahedral intermediates. They support further investigations on the use of transition-state and/or intermediate analogues as inhibitors of all β -lactamase classes.

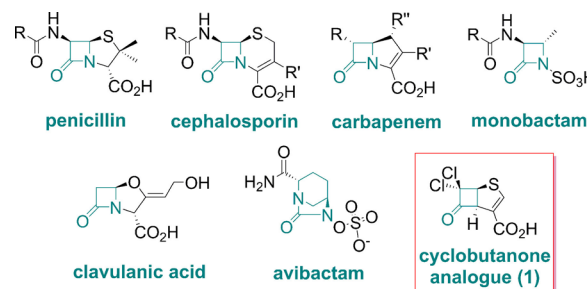


Figure 1. Structures of major classes of clinically used β -lactams, serine β -lactamase inhibitors, cyclobutanone analogue (1), and avibactam.

For more than 70 years, the β -lactams (Figure 1) have been the most widely used antibacterials. β -Lactam resistance is endem-

ic, substantially due to β -lactamases, which have two mechanistic classes—the nucleophilic serine β -lactamases (SBLs) and the zinc-dependent metallo- β -lactamases (MBLs) (Figure S1 in the Supporting Information).^[1] Combinations of a β -lactam and a β -lactam containing SBL inhibitor (e.g., clavulanic acid) have been clinically effective,^[2] however, the growing dissemination of new SBLs and MBLs, which are unaffected by such inhibitors, compromises this approach.^[3] Carbapenems are broad-spectrum antibacterials, which were once often used as last-line treatments. Their widespread use has led to the spread of SBL and MBL carbapenemases, especially in Gram-negative bacteria, for example, *Escherichia coli* and *Klebsiella pneumoniae*.^[4] Examples include both Class A and D SBLs and Class B MBLs (e.g., IMP-1, VIM-2, SPM-1, NDM-1). Avibactam has been introduced as a broad-spectrum SBL inhibitor and is the first clinically useful non- β -lactam β -lactamase inhibitor,^[5] however, it is a (poor) substrate of some SBLs and most MBLs.^[6] There is thus an unmet need for hydrolytically stable inhibitors active against both SBLs and MBLs.

One approach to obtain inhibitors active against the two mechanistically distinct classes of β -lactamases is to mimic the common tetrahedral intermediate (Figure 2A) or transition states pre- or succeeding it.^[7] Although increasing numbers of structures describe binding of hydrolyzed β -lactams to MBLs, progress in inhibitor development is hampered by the absence of structures describing interactions of MBLs with intact substrates/close analogues. We, and others, have been exploring cyclobutanone analogues of β -lactams as mechanistic probes and as templates for broad spectrum β -lactamase inhibition (Figure 2B). Early compounds, however, manifested only weak Class A SBL inhibition.^[8] Recently, we have found that cyclobutanone analogues of the penems and penams inhibit both

[a] Dr. M. I. Abboud, Dr. J. Brem, Prof. Dr. T. D. W. Claridge, Prof. Dr. C. J. Schofield
Department of Chemistry, University of Oxford
12 Mansfield Road, Oxford, OX1 3TA (UK)
E-mail: christopher.schofield@chem.ox.ac.uk

[b] Dr. M. Kosmopoulou, Dr. P. Hinchliffe, Dr. J. Spencer
School of Cellular and Molecular Medicine
University of Bristol, Medical Sciences Building
Bristol, BS8 1TD (UK)
E-mail: jim.spencer@bristol.ac.uk

[c] Dr. A. P. Krismanich, Dr. J. W. Johnson, Prof. Dr. G. I. Dmitrienko
Department of Chemistry, University of Waterloo
200 University Ave. W., Waterloo, Ontario, N2L 3G1 (Canada)
E-mail: dmitrien@uwaterloo.ca

Supporting information and the ORCID number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/chem.201705886>.

© 2018 Die Autoren. Veröffentlicht von Wiley-VCH Verlag GmbH & Co. KGaA. Dieser Open Access Beitrag steht unter den Bedingungen der Creative Commons Attribution License, die jede Nutzung des Beitrages in allen Medien gestattet, sofern der ursprüngliche Beitrag ordnungsgemäß zitiert wird.

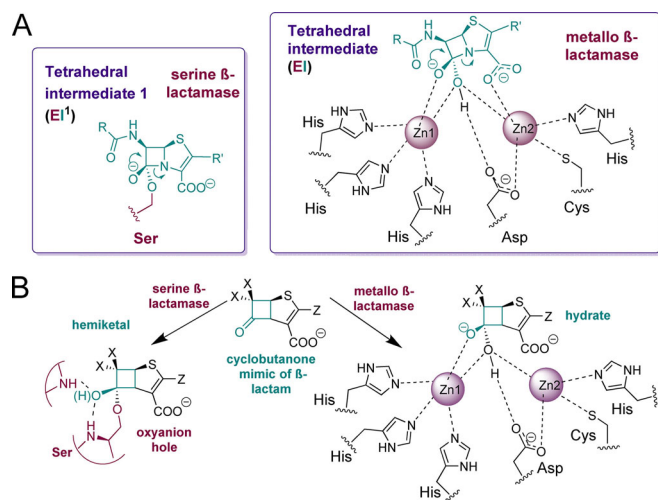


Figure 2. A Proposed binding modes of tetrahedral intermediates in the β -lactamase-catalyzed hydrolysis of a penem. B Cyclobutanones/penem analogues as potential broad-spectrum SBL and MBL inhibitors.

SBLs and MBLs.^[8a] We identified the cyclobutanone penem analogue **1** (Figure 1) to be the most potent compound tested against class A and C SBLs, and to have modest inhibition of the IMP-1 MBL.^[8a] However, although we obtained crystallographic evidence for SBL inhibition, involving binding of the cyclobutanone by a hemiketal to the nucleophilic serine,^[8a] no information has been available on how cyclobutanones inhibit MBLs.

The São Paulo MBL (SPM-1) is widely distributed in South America, Europe and North America, in the Gram-negative pathogen *Pseudomonas aeruginosa*.^[9] Like other B1 MBLs (NDM, VIM and IMP),^[10] SPM-1 has a binuclear zinc center, but has loop characteristics of the B2 MBLs, suggesting it is a B1/B2 hybrid (Figures S2 and 3 in the Supporting Information), which, consequently, may be challenging to inhibit. To test the hypothesis that cyclobutanones can act as tetrahedral intermediate analogues for MBLs, we initiated studies on the binding mode of **1** to SPM-1.

To study binding of **1** to SPM-1, we initially employed ^{19}F NMR (Figure S4 in the Supporting Information). SPM-1 was selectively labeled at residue 152 on its $\alpha 3$ region, which forms part of the active site cleft, using cysteine alkylation by 3-bromo-1,1,1-trifluoroacetone (BTFA) (Figure 3A).^[10–12] The ^{19}F NMR spectrum of labeled SPM-1 (SPM-1 Y152C*) manifests two peaks assigned as corresponding to “closed” (–83.3 ppm) and “open” (–72.4 ppm) conformations of the $\alpha 3$ loop (Figure S5).^[11a] Addition of known MBL inhibitors (e.g., isoquinoline derivatives, 1,10-*o*-phenanthroline) results in line broadening and chemical shift changes in the ^{19}F NMR of $\alpha 3$ variants.^[11a] By contrast, titration of **1** with SPM-1 Y152C* manifests only small effects on the SPM-1 Y152C* ^{19}F NMR spectra (Figure S5). We therefore employed a second BTFA-labeled mutant, SPM-1 Y58C*,^[11a] incorporating a ^{19}F label on the L3 loop that connects $\alpha 3$ and $\alpha 4$, and which is adjacent to the active site. The ^{19}F NMR spectrum of SPM-1 Y58C*^[11a] has one major peak (–83.3 ppm; Figure 3B). Addition of **1** (10 μM) causes a shift and line broadening, indicating **1** binds in the vicinity of Cys58

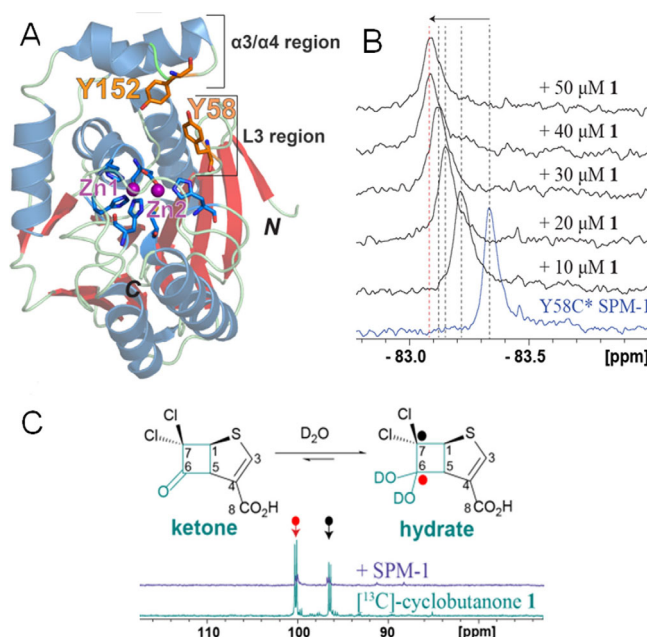


Figure 3. NMR reveals binding of cyclobutanone **1** to SPM-1. A) View from an SPM-1 crystal structure showing location of the ^{19}F labels. B) ^{19}F chemical shift changes for SPM-1 Y58C* (45 μM) on titration with cyclobutanone **1**. C) NMR spectroscopy implies binding of **1** to SPM-1 in its hydrated form. ^{13}C NMR spectrum of **1** (4.2 mM) (green) and ^{13}C NMR spectrum of **1** (4.2 mM) after addition of SPM-1 (0.84 mM) (purple). Circles highlight peaks assigned to the hydrated form of **1**. Assays were buffered with 50 mM Tris- D_{11} , pH 7.5, in 9:1 $\text{H}_2\text{O}:\text{D}_2\text{O}$.

in a fast-exchange manner relative to the NMR timescale. Monitoring the concentration dependence of ^{19}F chemical shift changes on titration of **1** into SPM-1 Y58C* enabled the K_D to be estimated as $22 \pm 7 \mu\text{M}$.

We then worked to obtain a structure of cyclobutanone **1** complexed to SPM-1 by soaking crystals with excess inhibitor. SPM-1 crystallized in its di-zinc “closed” form,^[10a] in which the $\alpha 3$ region folds over the active site, and in a previously unreported space group (P4₂22). The resolution extended to 1.7 Å for uncomplexed crystals and 2.38 Å for inhibitor soaked crystals (Table S1 in the Supporting Information). Electron density maps for the latter indicated clear $F_o - F_c$ difference density in the active site into which **1** was modelled as its hydrated form (Figure 4A). SPM-1, like other B1 MBLs, has a di-zinc ion active site with one zinc ion bound at a (normally tetrahedral) tri-histidine site (Zn1) and one in a trigonal bipyramidal site (Zn2) comprised of conserved Asp, Cys, and His residues (Figure S3). Wat1 “bridges” the zinc ions and is proposed to act as the “catalytic” nucleophile.^[10a] Relative to uncomplexed SPM-1 in the same crystal form (Table S1), a small (~ 0.5 Å) movement of Zn2 into a more solvent-exposed position in the active site is observed; however, the binding of **1** does not cause significant structural effects on the metal center with little change in Zn–Wat1; Zn1–Zn2 or Zn:protein ligand distances. Notable interactions made by **1** are in the vicinity of Zn2. The C4 carboxylate of **1** makes direct interactions with both Zn2 (2.48 Å distance) and Lys219 (2.91 Å; Figure 4C), a binding mode likely involved in substrate carboxylate binding in most B1 MBLs (Figure S7 in

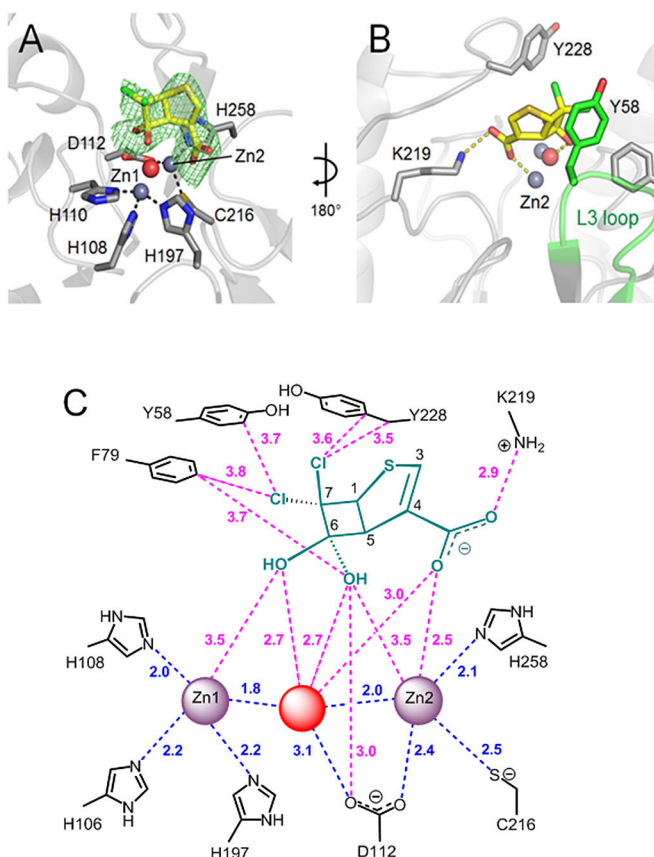


Figure 4. Binding mode of **1** to SPM-1 as observed crystallographically. A) Cyclobutanone **1** (yellow) binding to the SPM-1 active site of chain A (grey ribbon). $F_o - F_c$ density (green, contoured at 3σ) calculated from the SPM-1 model in the absence of ligand. Zinc ions and the bridging water/hydroxide (Wat1) are grey and red spheres, respectively. B) Interactions between SPM-1 and cyclobutanone **1** (yellow dashes). C) Interactions of **1** with SPM-1 in chain A. Distances between **1** (cyan) and the SPM-1 active site are in magenta. Distances between active site atoms are in blue. The red sphere represents the bridging water molecule or hydroxide. Numbers indicate distances in Å.

the Supporting Information).^[13] By contrast, **1** makes only weak interactions with Zn1, with the two oxygen atoms of the C6 ketone hydrate with Zn1-oxygen distances of 3.5 and 4.0 Å. In contrast to previous predictions about cyclobutanone binding to MBLs,^[14] the Zn-bridging Wat1 is clearly present, potentially interacting with both the C6 oxygens of **1** (2.7 Å; Figure 4C).

SPM-1 is distinguished from other B1 MBLs in possessing a “wall” of hydrophobic residues involving Phe57 and Tyr58 on its L3 loop, Phe79 on the loop connecting $\alpha 5$ and $\alpha 1$, and Phe151 and Tyr152 on the kinked $\alpha 3$ helix in the “closed” form, as well as Tyr228 on the opposite side of the active site cleft.^[10] Binding of **1** involves hydrophobic interactions with several of these, notably involving sandwiching of the bicyclic ring of **1** between the aromatic rings of Tyr58 (consistent with the ¹⁹F-NMR results, Figure 3B) and Tyr228, and edge-face interactions of **1** with Phe79 (Figure 4B). The Tyr152 side chain is more distant from **1**, with its OH group 6.5 (chain A) or 10.8 Å (chain B) away from the C7 atom of **1**, again consistent with the ¹⁹F NMR results (Figure S5). Notably, despite the clear presence of **1** at the active sites of both molecules in the asymmet-

ric unit, the conformation of the $\alpha 3$ loop differs substantially in hcm (Figure S8 in the Supporting Information); compared to the rest of the structure, this region is flexible (B-factors of 75 Å², crystallographic chain A and 64 Å², chain B) in support of the solution studies presented here and previously indicating $\alpha 3$ flexibility.^[10a, 11a] By contrast, we do not see evidence for changes in the L3 loop on binding of **1**. The larger ¹⁹F NMR chemical shift changes observed on binding of **1** to SPM-1 Y58C*, compared to SPM-1 Y152C*, can be rationalized by the closer proximity of residue 58 to the inhibitor binding site, notwithstanding the more extensive conformational changes observed in the $\alpha 3$ region crystallographically.

Test refinements with the ketone and hydrated forms of **1** yielded similar statistics. We therefore used NMR to investigate the behavior of **1** in solution both in the presence and absence of SPM-1. We synthesized **1** in which the C6 (ketone) and adjacent C7 dichlorinated carbon atoms were labeled with [^{13}C]. Under the conditions of the binding studies, [^{13}C]-**1** was found to exist (almost) entirely as its hydrate (consistent with previous work),^[8a] as indicated by the ^{13}C chemical shift (102 ppm) for C6 and (98.7 ppm) C7, $J_{\text{CC}} = 38$ Hz. Binding of **1** to SPM-1 under these conditions was confirmed by ^{13}C NMR, in which the peaks corresponding to hydrated **1** were reduced on addition of SPM-1 (Figure 3C). Addition of SPM-1 to the sample yielded no change in the chemical shifts of either the C6 or C7 peaks, with no appearance of a peak around 190 ppm indicative of a ketone at C6. These results support the proposal that **1** is bound to SPM-1 in its hydrated form, although we cannot exclude initial binding of the ketone form.

It is instructive to consider our results in the context of possible modes of β -lactam binding, as our work represents the closest stable analogue of an intact β -lactam for which an MBL complex structure is currently available. The structure implies substrate binding includes important interactions of the substrate carboxylate with Zn²⁺ and the adjacent Lys219, as observed for hydrolyzed β -lactams^[13] (Figure S7 in the Supporting Information) and β -lactam analogues^[15] (Figure S9), as well as interactions with hydrophobic elements around the active site (Figure 4). The structure supports the involvement of these interactions in binding intact β -lactam substrates, and, thus, in the formation of early-stage complexes in catalysis.

It is notable that the complex with **1** does not feature strong interactions of the C6 oxygen atoms with Zn1. The Zn1 site is proposed to polarize the β -lactam amide and participate in activation of the "hydrolytic" water (Wat1).^[16] As such, it is proposed that binding of an intact β -lactam triggers dissociation of Wat1 from Zn2 to generate a "terminal" hydroxide nucleophile on Zn1. The tetrahedral species formed by reaction of Wat1 with the β -lactam carbonyl is stabilized by Zn1 binding, as supported by structural analysis of MBLs complexed with cyclic boronates, which are tetrahedral intermediate analogues (Figure S9 in the Supporting Information).^[15] In the hydrated cyclobutanone, the C6 oxygen atoms are likely protonated, possibly reducing affinity for Zn1 and instead favoring interaction with Wat1. Further, approach of the non-polar C5–H bond of **1** to Zn2, which may be required to bring Zn1 and

the C6 oxygen atoms into proximity, may be disfavored compared to that of β -lactam derived amide nitrogen.

Inhibition of SBLs by cyclobutanones involves formation of a hemiketal linkage through reaction of the nucleophilic serine with the ketone and binding of the resultant tetrahedral species in the "oxanion hole".^[8a] By contrast to the stabilization of tetrahedral intermediates by SBLs, MBLs are proposed to preferentially stabilize the ring-opened anionic intermediates formed after addition of the water/hydroxide (Wat1) nucleophile to the β -lactam carbonyl, but prior to proton transfer to the lactam-derived nitrogen (Figure S1 in the Supporting Information). Interaction of Zn²⁺ with the anionic nitrogen of such species is proposed to be important in intermediate stabilization.^[16] Our results, however, show that the SPM-1 active site can bind with reasonable affinity to a species (i.e., a hydrated cyclobutanone) whose tetrahedral carbon (C6) atoms render it closely (though not perfectly) analogous to the oxanion intermediate. Taking into consideration the activity of cyclobutanones and boronates against all classes of SBLs,^[8a,17] these results indicate that structures mimicking the tetrahedral oxanion merit investigation as starting points for potent inhibitors that are hydrolytically stable and effective against both SBLs and MBLs.

Acknowledgements

We thank Diamond Light Source for access to beamline I03 (proposal number MX313) and support. J.S., M.I.A., J.B., and C.J.S. are supported by the MRC (G1100135), the SWON alliance, and the Wellcome Trust, G.I.D. by the Canadian Institute of Health Research (FRN114046 and FRN106532), and M.I.A. by the Sir Hans Krebs Memorial Award. M.K., P.H., and J.S. are supported by the National Institute of Allergy and Infectious Diseases (NIH, R01AI100560); the content is solely the responsibility of the authors and does not necessarily represent views of the NIH.

Conflict of interest

The authors declare no conflict of interest.

Keywords: antimicrobial resistance • β -lactam analogues • β -lactamases • cyclobutanones • transition state analogues

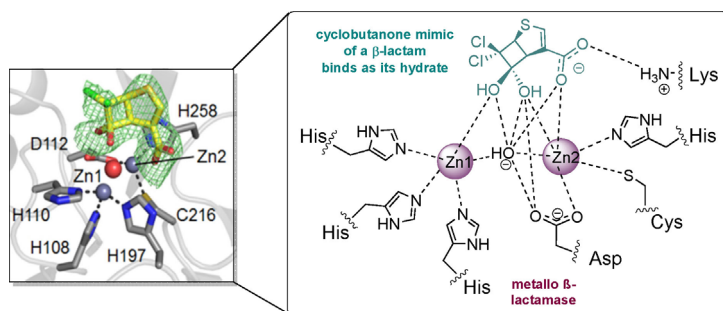
- [1] C. Bebrone, *Biochem. Pharmacol.* **2007**, *74*, 1686–1701.
- [2] K. A. Toussaint, J. C. Gallagher, *Ann. Pharmacother.* **2015**, *49*, 86–98.
- [3] T. R. Walsh, M. A. Toleman, L. Poirel, P. Nordmann, *Clin. Microbiol. Rev.* **2005**, *18*, 306–325.
- [4] A. Y. Peleg, D. C. Hooper, *N. Engl. J. Med.* **2010**, *362*, 1804–1813.
- [5] a) D. Y. Wang, M. I. Abboud, M. S. Markoulides, J. Brem, C. J. Schofield, *Future Med. Chem.* **2016**, *8*, 1063–1084; b) D. Y. Wang, M. I. Abboud, M. S. Markoulides, J. Brem, C. J. Schofield, *Med. Lett. Drugs Ther.* **2015**, *57*, 79–80.
- [6] M. I. Abboud, C. Damblon, J. Brem, N. Smargiasso, P. Mercuri, B. Gilbert, A. M. Rydzik, T. D. Claridge, C. J. Schofield, J. M. Frere, *Antimicrob. Agents Chemother.* **2016**, *60*, 5655–5662.
- [7] K. W. Yang, L. Feng, S. K. Yang, M. Aitha, A. E. LaCuran, P. Oelschlaeger, M. W. Crowder, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5855–5859.
- [8] a) J. W. Johnson, M. Gretes, V. J. Goodfellow, L. Marrone, M. L. Heynen, N. C. Strynadka, G. I. Dmitrienko, *J. Am. Chem. Soc.* **2010**, *132*, 2558–2560; b) J. W. Johnson, D. P. Evanoff, M. E. Savard, G. Lange, T. R. Ramadhar, A. Assoud, N. J. Taylor, G. I. Dmitrienko, *J. Org. Chem.* **2008**, *73*, 6970–6982; c) A. C. Stewart, I. J. Clifton, R. M. Adlington, J. E. Baldwin, P. J. Rutledge, *ChemBioChem* **2007**, *8*, 2003–2007.
- [9] a) A. P. Carvalho, R. M. Albano, D. N. de Oliveira, D. A. Cidade, L. M. Teixeira, A. Marques Ede, *Microb. Drug Resist.* **2006**, *12*, 103–108; b) K. L. Hopkins, D. Meunier, J. Findlay, N. Mustafa, H. Parsons, R. Pike, L. Wright, N. Woodford, *J. Med. Microbiol.* **2016**, *65*, 696–697; c) A. E. Salabi, M. A. Toleman, J. Weeks, T. Bruderer, R. Frei, T. R. Walsh, *Antimicrob. Agents Chemother.* **2010**, *54*, 582.
- [10] a) J. Brem, W. B. Struwe, A. M. Rydzik, H. Tarhonskaya, I. Pfeffer, E. Flashman, S. S. van Berkel, J. Spencer, T. D. Claridge, M. A. McDonough, J. L. Benesch, C. J. Schofield, *Chem. Sci.* **2015**, *6*, 956–963; b) T. A. Murphy, L. E. Catto, S. E. Halford, A. T. Hadfield, W. Minor, T. R. Walsh, J. Spencer, *J. Mol. Biol.* **2006**, *357*, 890–903.
- [11] a) M. I. Abboud, P. Hinchliffe, J. Brem, R. Maccsics, I. Pfeffer, A. Makena, K. D. Umland, A. M. Rydzik, G. B. Li, J. Spencer, T. D. Claridge, C. J. Schofield, *Angew. Chem. Int. Ed.* **2017**, *56*, 3862–3866; *Angew. Chem.* **2017**, *129*, 3920–3924; b) A. M. Rydzik, J. Brem, S. S. van Berkel, I. Pfeffer, A. Makena, T. D. Claridge, C. J. Schofield, *Angew. Chem. Int. Ed.* **2014**, *53*, 3129–3133; *Angew. Chem.* **2014**, *126*, 3193–3197; c) W. H. Huestis, M. A. Raftery, *Biochem. Biophys. Res. Commun.* **1978**, *81*, 892–899.
- [12] L. J. González, D. M. Moreno, R. A. Bonomo, A. J. Vila, *PLoS Pathog.* **2014**, *10*, e1003817.
- [13] H. Feng, J. Ding, D. Zhu, X. Liu, X. Xu, Y. Zhang, S. Zang, D. C. Wang, W. Liu, *J. Am. Chem. Soc.* **2014**, *136*, 14694–14697.
- [14] P. Devi, P. J. Rutledge, *ChemBioChem* **2017**, *18*, 338–351.
- [15] J. Brem, R. Cain, S. Cahill, M. A. McDonough, I. J. Clifton, J. C. Jimenez-Castellanos, M. B. Avison, J. Spencer, C. W. Fishwick, C. J. Schofield, *Nat. Commun.* **2016**, *7*, 12406.
- [16] a) M. I. Page, A. Badarau, *Bioinorg. Chem. Appl.* **2008**, 576297; b) A. I. Karsiotis, C. F. Damblon, G. C. Roberts, *Metallomics* **2014**, *6*, 1181–1197.
- [17] S. T. Cahill, R. Cain, D. Y. Wang, C. T. Lohans, D. W. Wareham, H. P. Oswin, J. Mohammed, J. Spencer, C. W. Fishwick, M. A. McDonough, C. J. Schofield, J. Brem, *Antimicrob. Agents Chemother.* **2017**, *64*, e02260-16;

Manuscript received: December 12, 2017

Accepted manuscript online: December 17, 2017

Version of record online: ■■■■■, 0000

COMMUNICATION



Mimicking intermediates: Solution and crystallographic studies on the interaction of a cyclobutanone penem analogue with the clinically important MBL

SPM-1 are described. The results inform on how metallo- β -lactamases bind substrates and stabilize tetrahedral intermediates.

Enzyme Catalysis

M. I. Abboud, M. Kosmopoulou, A. P. Krismanich, J. W. Johnson, P. Hinchliffe, J. Brem, T. D. W. Claridge, J. Spencer, C. J. Schofield,* G. I. Dmitrienko**



Cyclobutanone Mimics of Intermediates in Metallo- β -Lactamase Catalysis

